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Genome sequencing and transcriptome analysis of Geotrichum citri-aurantii on citrus reveal the potential pathogenic- and guazatine resistance-related genes

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Abstract

Background: Citrus grow in more than 100 countries and is one of the most produced fruit genus. Sour rot, caused by Geotrichum citri-aurantii , is a major postharvest disease of citrus and it causes economic losses. In recent years, the disease had a rising trend year by year. In this study, the genome sequence of G. citri-aurantii and transcriptome sequence of pathogenic- and guazatine resistance were sequenced with a view to explore the potential pathogenic mechanism and drug resistance mechanism of G. citri-aurantii on citrus.

Results: We sequenced a high-quality genome sequence of G. citri-aurantii by SMRT. This sequence encodes 6,783 predicted genes of the 28.1-Mb G. citri-aurantii genome. Approximately 5.43 Gb of clean data were obtained after Hi-C sequencing, and a 27.94-Mb genomic sequence was positioned to the 10 chromosome groups after Hi-C assembly , accounting for 99.43% of the previously measured G. citri-aurantii genome. In the process of studying pathogenic mechanisms, the content of polygalacturonase (PG) and polymethylgalacturonase (PMG) was considerably increased in the Newhall navel orange infected by G. citri-aurantii. Then, three polygalacturonase (PG) genes (EVM0005942, EVM0004416, EVM0002276) related to pathogenicity were identified and the expression level was significantly increased during the infection by quantitative RT-PCR. Additionally, G. citri-aurantii is only sensitive to the chemical fungicide guazatine. Massive guazatine use has led to evolution of the wild G. citri-aurantii in citrus-producing areas. Owing to its uniqueness, RNA sequencing analysis of guazatine-resistance showed that the guazatine-resistance of G. citri-aurantii is may related to two ABC transporter family genes, six MFS transporter family genes and two MATE transporter family genes.

Conclusions: We found three polygalacturonase (PG) genes related to pathogenicity and ten genes related to guazatine-resistance from molecular level. Our research may provide novel insights into the effective control of this pathogen.

Keywords: Geotrichum citri-aurantii , citrus, genome, pathogenicity, guazatine, drug resistance

Background

Sour rot, caused by *Geotrichum citri-aurantii* (Ferraris), is one of the most important fungal diseases of citrus fruits, affecting all species and cultivars in the world[1]. After being first reported in 1917, this disease has been frequently reported around the world[2]. During the period of harvest, storage, transport and handling, citrus fruits are exposed to the *G. citri-aurantii* pathogen and can be infected through wounds[3], especially during rainy seasons.

Despite the severe damage caused by *G. citri-aurantii*, its infection mechanism and genetic information have not been thoroughly elucidated. Many researchers have tried to control citrus sour rot by physical control, chemical control and biological control[4-7]. However, no suitable methods have been found to

effectively control the occurrence and spread of sour rot disease in actual production. Thus, obtaining genome information is one of the main strategies to elucidate pathogen infection mechanisms.

Furthermore, guazatine is the only chemical fungicide that can control sour rot effectively[8]. As a broad-spectrum bactericidal agent, guazatine plays a role in the biosynthesis of fungal esters, cell membrane function, and inhibiting spore germination[9], germ tube elongation and adherent cells and hyphae formation. In recent years, the incidence of sour rot has increased in many citrus-producing areas of China. Simultaneously, the use of the guazatine concentration in citrus-producing areas has increased annually. The above-mentioned reports imply that *G. citri-aurantii* could adapt to the changing environment flexibly. Therefore, the *G. citri-aurantii* genome may be helpful for understanding the anti-drug mechanism of *G. citri-aurantii*, and it is more conducive to finding an effective method to control sour rot instead of traditional fungicides.

In previous studies, ABC, MFS and MATE (multidrug and toxic compound extrusion) transporter family genes play a crucial role in the process of fungal resistance. Two ABC transporter family genes, PMR1 and PMR5, mediate DMI fungicide efflux. The MFS transporter family genes also contributes to fungicide resistance in *Penicillium digitatum*[10]. The toxic efflux system consisting of PMR1 is directly involved in DMI resistance of fungi[11]. Multidrug efflux transporters play important roles in the regulation of fungal resistance to many DMI drugs. At present, in terms of the primary structure and special energy coupling mode, multidrug efflux transporters are categorized into five families. The MATE family is one of these families[12].

Sequencing of *G. citri-aurantii* genome and transcriptome open the possibility of identify genes that are related to pathogenic mechanism and guazatine-resistance mechanism. So far, the genome of *G. citri-aurantii* is not recently released.

In this study, we aimed to get which genes in *G. citri-aurantii* genome associated with pathogenic and guazatine-resistance. Extraordinary, PG could promote infection by some pathogens according to transforming the mechanical strength and rheological properties of the cell wall[13]. ABC transporter family genes, MFS transporter family genes and MATE family genes were proved to evolve resistance to guazatine in *P.digitatum* genome. For this, we integrated genome sequencing and transcriptome data to study the expression of pathogenic- and drug resistance-related genes.

Results

The *G. citri-aurantii* genome sequencing, assembly and annotation.

The genome of *G. citri-aurantii* was sequenced by PacBio RS II. A 20-Kb library was constructed for genomic DNA. After filtering the original data, a total of 2.54 Gb of high-quality sequencing data was obtained (Fig 1A). Assembly led to a genome size of 28.10 Mb (28,101,572bp) with approximately 90x

coverage, divided into 29 scaffolds with a minimum size of 1 Mb, as well as an overall G + C content of 38.6% (Table 1). And an overall repeat content of 17.93% of the genome was discovered through RepeatMasker analysis and PASTEClassifier classification. Some characteristics of *G. citri-aurantii* genome are shown in Table 1.

6,783 genes were predicted by BRAKER software, with an average length of 2,058 bp, a gene density of 241 genes/Mb and a total of 46.8% of the genome covered by protein-coding genes. There is an average exon and intron length of 1,203bp and 111bp.

Ten chromosome groups can be clearly distinguished according to the evaluation of HI-C assembly results (Fig. 1C) and displayed by circus plot (Fig. 1D). Within each group, the intensity of the interaction at the diagonal position is higher than the position of the nondiagonal line, and the results indicated that the interaction between adjacent sequences (diagonal positions) is higher in the Hi-C assembled chromosome but not in nonadjacent sequences (nondiagonal position), which is consistent with the principle of Hi-C helper genome assembly. After Hi-C assembly, the genome sequence length of 27.94 Mb with 193x coverage was mapped to the chromosome, accounting for 99.43% of the previously measured *G. citri-aurantii* genome. In the sequence mapped to the chromosome, the sequence length and direction can be determined to be 27.58 Mb, accounting for 98.63% of the total length of the sequence located on the chromosome (Table 1).

In the genome structure, TEs and repetitive DNA sequences play important roles in the evolution of fungi[14]. The total repeat sequences of 5,039,960 bp (5 M) were identified, accounting for 17.93% of the *G. citri-aurantii* total genome, including LTR retrotransposon, DNA transposon, transposon tandem and other unclassified repeat sequences (Fig. 2A). The number of repeats reached 11,476. Interestingly, Rest of genome are the most repeating sequence (82.17%). Notably, LINE and LTR account for 5.1 and 2.31%, respectively.

Recently, DNA methylation has also been reported in the class Dothideomycetes[15]. In *G. citri-aurantii* genome, 171,537 m4C (4-methyl-cytosine) and 11,765 m6A (6-methyl-adenosine) were identified (Fig. 2B). Most of the categorized DNA methylations are m4C. However, m6A DNA methylations occur with high frequency in the regions of repetitive elements compared with m4C (Fig. 2C).

Using the predicted protein sequence of the gene to perform BLAST alignment with functional databases, such as the TCDB database and the PHI database, 170 TCDB and 1,933 PHI were predicted. Then, 196 CAZyme and 1,270 Transmembrane proteins were found through analysis of protein sequences of all predicted genes (Fig. 2D). In *G.citri-aurantii* genome, The protein containing the transmembrane helix was removed from the predicted 339 proteins containing the signal peptide, and the remaining 158 proteins were secreted proteins. Through domain calling analysis, PF14295.4 (n=6) is the most abundant domain, which mediates protein/protein interactions.

Comparative genomic analysis

The evolutionary relationship of *G. citri-aurantii* and other fungi species was analyzed using a group of phylogenetic backbone genes of the fungi. Phylogenetic analysis revealed that *G. citri-aurantii* is evolutionarily close to Galactomyces citri-aurantii, a plant pathogen that has been found on citrus fruit or in the soil of citric fruit orchards (Fig. 3A). In addition, *G. citri-aurantii* is also close to the other two fungi of Dipodascaceae sp. and *Geotrichum candidum* (Fig. 3A). To date, in all fungi in the phylogenetic tree of *G. citri-aurantii*, only the genome of *Geotrichum candidum* has been published. Synteny analysis of the *G. citri-aurantii* genome and *G. candidum* genome revealed that the *G. citri-aurantii* genome displays different synteny than those fungi (Fig. 3B–C).

The potential pathogenic related genes analysis of *G. citri-aurantii*.

The plant cell wall is the first barrier of defense against pathogens. Nearly all fungi, particularly necrotrophic pathogens, must initially secrete PGs to dissolve pectin and related components of plant cell wall[16-18]. In vitro, *G. citri-aurantii* was induced to produce five cell wall-degrading enzymes, polygalacturonase (PG), polymethylgalacturonase (PMG), cellulase (Cx), polygalacturonic acid transeliminase (PGTE) and pectinmethyltranseliminase (PMTE) (Fig. 4). PG and PMG have higher activity, and both PGTE and PMTE enzyme activities are less than 1 U/mg (Fig. 4A). However, in the fruit wounds after culture for 72 h, the activities of only four enzymes, PG, PMG, PGTE and PMTE, were detected. The activity of Cx was zero (Fig. 4B). Importantly, the PG activity of the fruit pathological site was significantly higher than that of the healthy part, which is 6.8 times that of the healthy part, and the PMG activity is 1.6 times that of the healthy part.

In *G.citri-aurantii* genome, three PG enzymes have been discovered, and we identified the genes that produce these three proteins: EVM0005942, EVM0004416 and EVM0002276. The results of quantitative experiments were the same as those of the transcriptome (Fig. 4C). After the citrus was infected by *G. citri-aurantii*, the expression levels of these three enzymes increased greatly, indicating that these three enzymes may play a critical role in the process of *G. citri-aurantii* infection of citrus.

Transcriptome analysis on the guazatine-resistance of *G. citri-aurantii*.

The guazatine-resistance transcriptome data was repeatable between samples (Fig. 5C). There were 714 DE genes (FDR=2 and FC = 0.05) between AY-1 and AY1-68 of *G. citri-aurantii* before guazatine treatment and were used as cutoff values (Fig.5E). Among these genes, 385 genes were upregulated, and 329 genes were downregulated. The results indicated that the gene expression pattern was changed significantly in the resistant strains. After guazatine treatment, there were 226 DE genes in AY1-68 that contained 52 downregulated and 174 upregulated DE genes. In addition, 226 DE genes belonging to AY1-68 were uniquely associated with the resistance of AY1-68 to guazatine. There were 108 DE genes between G1 and G3 after guazatine treatment, and they were associated with the resistance of AY1-68 to the drug response of guazatine.

All 1146 DE genes and selected genes were classified by Gene ontology (GO) consortium analysis (Fig. 5F). Compared with the wild strain AY-1, the drug-resistant strain AY1-68 could increase its resistance several hundredfold. It is suspected that some of the cellular component organization of this strain has changed to some extent. Simultaneous sequencing revealed that 80% of these genes have increased expression levels of the transcriptome data. These results revealed that the cellular component of the drug-resistant strain AY1-68 changed.

To identify genes related to the responses of *G. citri-aurantii* to the antifungal drug guazatine and the high resistance of *G. citri-aurantii*, differentially expressed genes of the ABC transporter family and MFS transporter family were further analyzed. The two genes (EVM0003677, EVM0005437) of the ABC transporter family and six transporter genes (EVM0000087, EVM0000766, EVM0001093, EVM0003101, EVM0004095, EVM0005235) of the MFS transporter

family received more attention. In this study, RT-qPCR was used to validate the expression levels of eight transporter genes (Fig. 6A, C). these results indicate that overexpression of these genes may enhance the drug resistance of *G. citri-aurantii*.

Transcriptome data analysis revealed that seven MATE transporters (EVM0000063, EVM0002368, EVM0001080, EVM0002368, EVM0003579, EVM0005976, EVM6710) were related to drug resistance, and these transporter genes were not reported before this study. In the guazatine-resistant strain AY1-68, the expression levels of two MATE genes (EVM0000063, EVM0002368) were upregulated compared with the guazatine-susceptible strain AY-1 (Fig. 6B). The other five MATE genes had decreased expression. Notably, these MATE transporters were normal in AY1-68 after guazatine treatment. These results indicate that two ABC genes and six MFS genes of *G. citri-aurantii* were activated after guazatine treatment. However, two MATE genes may play a decisive role in enhancing drug resistance.

Discussion

In postharvest diseases of citrus, the incidence of sour rot caused by *G. citri-aurantii* has increased annually in various producing areas of China [9]. Due to the large-scale use of the fungicide guazatine, the proportion of *G. citri-aurantii*-resistant mutant strains has also increased significantly. In our study, the *G. citri-aurantii* genome of the economically important fungi was sequenced by SMRT for the first time.

The transcripts were analyzed in accordance to the *G. citri-aurantii* genome. And all the functions in the genome were also identified in the transcriptome. From the total amount of genes, 3 PGs genes were may related to pathogenesis and 1146 genes were likely to enhance the resistance of sour rot to guazatine. Our preliminary work did not reveal the complex mechanisms of pathogenesis and the guazatine resistance. However, it may lay a foundation for future research on molecular mechanisms.

Epigenetics is currently at the forefront in the identification of pathogen-induced modifications within host plants[19]. However, DNA methylation is an important epigenetic mark associated with plant immunity, but little is known about its roles in fungi infection of citrus[20]. The most studied type of DNA methylation in fungi is m5C, while in prokaryotic cells, it is m4C and m6A. Recently, 171,915 m4C (4-methyl-cytosine) and 11,784 m6A (6-methyl- adenosine) (Fig. 2B) in the *G. citri-aurantii* genome were identified by SMRT for the first time. Surprisingly, large numbers of m4C were observed in *G. citri-aurantii*. The m4C modification occurs with a higher frequency in the region of repetitive elements in the *G. citri-*

aurantii genome (Fig. 2C), and the results indicate that m4C may be involved in the transposition of the transposons[19]. Our results also suggest that m4C may be the focus of future research on sour rot.

PG was first obtained from pectinase produced by pathogenic fungi by Cooper. PG is thought to act by degrading homologous polygalactonic acid regions in plant cell walls[21]. PG is associated with fungal pathogenicity and toxicity by causing tissue degradation and protoplast death[22]. We found three PG genes in the *G. citri-aurantii* genome, and RT-qPCR results verified that the expression of three PG genes increased. These data further support the hypothesis that PG can promote *G. citri-aurantii* to cause citrus disease.

In drug resistance research of *Penicillium digitatum*, further research provided evidence that transporter genes from the ABC transporter family and the MFS transporter family have also contributed to fungicide resistance. Two ABC transporter family genes, PMR1 and PMR5, mediate DMI fungicide efflux[23]. MATE (multidrug and toxic compound extrusion) is a multidrug efflux transporter that plays important roles in intrinsic and acquired resistance in many bacteria and in the regulation of fungi resistance to many DMI drugs[24]. In the study of *G. citri-aurantii* resistance, the expression of two genes from the ABC transporter family, six genes from the MFS transporter family and two genes from the MATE transporter family increased in RNA-seq data. These results attracted our attention to study the resistance of *G. citri-aurantii* to guazatine.

Conclusions

In summary, we report a complete genome sequence of *G. citri-aurantii* using the SMRT sequencing method. Then the genome was assembled and annotated. These data not only assist us in finding 17.93% repetitive elements but also in discovering 171,915 m4C and 11,784 m6A. Using genomic component analysis, gene function annotation and classification, we predicted CAZymes, PHI and TCDB associated with the pathogenicity of sour rot. Additionally, we identified three PG genes related to pathogenesis of *G. citri-aurantii* and some ABC, MFS and MATE genes related to guazatine resistance. Our work also lays the foundation for future research on this important citrus postharvest disease.

Methods

Fungal strains, culture conditions and DNA extraction.

A wild-type strain of *G. citri-aurantii* (AY-1) was isolated from Newhall navel orange in Anyuan city, Jiangxi Province of China. The single-sporing of *G. citri-aurantii* was first obtained by separation and purification by Zhe Wang (2012). Finally, the obtained fungi were formulated into a spore suspension and inoculated onto healthy Newhall navel oranges according to the Koch postulate[25]. The strain is identified as *G. citri-aurantii* based on the molecular identification of the ITS universal primer and the Blast alignment on the NCBI.

To obtain the purer mycelia, *G. citri-aurantii* was cultured on PDA media with a layer of clear cellophane at 28℃ for 5d. Genomic DNA of *G. citri-aurantii* was extracted by using a modified cetyltrimethylammonium bromide (CTAB) method[26].

Genome sequencing, assembly and chromosomal localization by Hi-C.

The genome of *G. citri-aurantii* was sequenced by the single molecule real-time (SMRT) sequencing method with P6-C4 of the latest upgraded chemical reagents. Then, the relationship between the whole chromatin DNA in the whole genome was studied by high-throughput sequencing technology with the Illumina HiSeq platform and Hi-C (high-through chromosome conformation capture) technology, which was combined with the biological information analysis method in Biomarker Technologies (Beijing, China). The sequencing read length is up to PE150.

Genomic component analysis.

A repetitive sequence library of *G. citri-aurantii* was constructed based on LTR_FINDER, MITE-Hunter, RepeatScout and PILER-DF software. Finally, the repeated sequence prediction of the sequencing data was performed based on the constructed repetitive sequence database using RepeatMasker software.

Gene prediction was performed using de novo prediction, homologous species prediction, and reference-based transcript-based assembly.

An increasing number of studies have found that noncoding RNA plays a significant role in the process of microbial infection. Blastn was used for genome-wide alignment to identify rRNA based on the Rfam database. tRNAscan-SE was used to identify tRNA.

The functional pseudogenes can be recombined by gene conversion into the respective expression site to create a new variant of each gene. Therefore, the study of pseudogenes is particularly important when gene function is studied. Through BLAST alignment, a homologous gene sequence (possible gene) is searched for in the genome. GeneWiseis used to find immature stop codons and frameshift mutations in the gene sequence to obtain pseudogenes.

Genomic function annotation.

To obtain gene function annotation, the predicted gene sequences were BLAST-matched with functional databases, such as KOG, KEGG, Swiss-Prot, TrEMBL, and Nr. According to the results of the Nr database comparison, the functional annotation of the GO database is performed using the software Blast2GO.

Protein-encoding genes were annotated by a combination of three databases: The Transporter Classification Database (TCDB), Pathogen Host Interactions (PHI) and CAZyme.

Whole-genome DNA methylation analysis.

Based on the results of the de novo assembly, the IPD ratio (the primary metric for kinetic information) was calculated based on the raw data obtained by SMRT sequencing to identify DNA methylation sites[27].

Synteny analysis and Phylogenetic analysis

The relevant reference sequences of the ITS sequences of strain AY-1 were downloaded in GenBank using ClustalX 2.0.10 software. Sequence alignment was performed using MUSCLE, and the phylogenetic tree was generated by MEGA 5.0 using a UPGMA method. *G. citri-aurantii* and *Geotrichum candidum* were analyzed using GATA.

Extraction and activity determination of the cell wall degrading enzyme of *G. citri-aurantii*

Five microliters of the spore suspension (1.0×10^6 spores/mL) of *G. citri-aurantii* was cultured on PDA medium (potato 200 g, sucrose 20 g, agar powder 15 g, distilled water 1 L) at 28℃ for 3 d. The inoculating needle was used to pick the mycelial lump of the colony into the modified Marcus culture medium (KNO_3 2.0 g, KCl 0.5 g, $FeSO_4$ 0.01 g, K_2HPO_4 1.0 g, $MgSO_4{\cdot}7H_2O$ 0.5 g, VB1 0.1 mg, L-asparagine 0.5 g, citrus pectin or carboxymethylcellulose sodium salt 10.0 g, distilled water 1 L, pH 5.0) to be grown at 28℃ and 150 rpm shaking (cellulase culture for 10 d, other enzyme culture for 6 d). This step also induces enzyme production.

The hyphae and impurities in the Marcus medium were filtered off by two layers of gauze, and the filtrate was centrifuged twice at 10000 rpm for 15 min; then, the supernatant was combined. Next, $(NH4)_2SO_4$ was gradually added to the supernatant enzyme solution, slightly vortexed to dissolve, and finally brought to 60% saturation (25℃). The mixture was allowed to sit at 4℃ for 5 h or overnight. The precipitate was obtained by centrifugation at 12 000 rpm for 15 min. Polygalacturonase (PG), polymethylgalacturonase (PMG) and cellulase (Cx) were dissolved in 20 mL of 50 mmol/L acetic acid-sodium acetate buffer (pH=5.0) and dialyzed in the buffer. PGTE and PMTE were treated with 20 mL of 50 mmol/L glycine-NaOH buffer (pH =9.0), and the precipitate was dissolved and dialyzed in the buffer. The dialysate was changed once every 12 h for a total of 3 dialysis cycles, and the enzyme purified by dialysis was stored at -20℃.

The spore suspension (1.0×106 spores/mL) of *G. citri-aurantii* was inoculated into Newhall navel orange and stored for 72 h at 25℃ and 95% RH. The peel sample was taken down with a scalpel around the diseased part of the fruit. In the same way, five cell wall degrading enzymes were extracted and purified.

According to the absorbance value (OD value) of the reducing sugar released by the enzyme reaction, the activity of polygalacturonase (PG), polymethylgalacturonase (PMG), Cellulase (Cx), polygalacturonic acid

transeliminase (PGTE) and pectinmethyltranseliminase (PMTE) were measured by a UV2450 spectrophotometer in vitro and in vivo. However, in vivo, the sampling time points were set to 36 h, 48 h, 60 h, 72 h, 84 h and 96 h.

Transcriptome analysis and quantitative RT-PCR

For pathogenic transcriptome analysis, *G. citri-aurantii* was cultured on cellophane on PDA medium at 28℃ for 10 d. The treatment group of *G. citri-aurantii* was grown in Newhall navel orange at 28℃ for 10 d. Then, the hyphae and spores were scraped gently using a sterile spoon from the cellophane and the navel orange surface. All samples were frozen at -80℃ before transcriptome sequencing. Each treatment had three biological replicates.

The spores and hyphae of *G. citri-aurantii* were used as a control group for drug resistance transcriptome analysis. *G. citri-aurantii* (AY1-68) is a mutant and is resistant to guazatine with an EC_{50} value of 6.3793 mg/L. However, *G. citri-aurantii* is sensitive to guazatine with an EC_{50} value of 0.0487 mg/L. *G. citri-aurantii* and the strain AY1-68 were cultured on PDA medium at 28℃. Then, 20 µl of a conidial suspension (10^6 spores ml-1) of *G. citri-aurantii* and strain AY1-68 were cultured in 100 ml PDB medium (extract of 200 g potato boiled water and 20 g dextrose) at 25℃ for 72 h, respectively. Then, guazatine (6.3793 mg/L) was added to PDB with shaking for an additional 6 h after being cultured at 28℃ for 48 h. Other groups were added with the same volume of sterile water as a control. The experiment was repeated three times. The samples were as follows: dE6-MI (*G. citri-aurantii* before the guazatine treatment); PdF6-MI (the strain AY1-68 before the guazatine treatment); PdF6-NI (the strain AY1-68 after the guazatine treatment). The mycelia were filtered and washed several times using double distilled water. All samples were frozen at -80℃ before transcriptome sequencing. Each treatment had three biological replicates.

The downloaded data were filtered to obtain clean data, sequence alignment with the specified reference genome[28], the obtained mapping data, insert length test, randomness test and other library quality evaluation[29]. Structural level analysis of alternative splicing analysis, new gene discovery, and genetic structure optimization were performed on the basis of the clean data. Bioinformatics analysis was performed based on the expression levels of genes in different samples or different sample groups, such as differential expression analysis[30], functional expression annotation of differentially expressed genes, and functional enrichment.

Total RNA was extracted using RNAiso Plus (TaKaRa Biotech. Co., Wuhan, China) according to the manufacturer's protocol. First-strand cDNA was prepared by NovoScript Plus All-in-one 1st strand cDNA Synthesis SuperMix (gDNA Purge) (Novoprotein, Beijing, China) following the manufacturer's protocol. qRT-PCR was performed using a BIO-RAD CFX96 q-PCR system with SYBR Green I fluorescent dye detection. The mRNA abundance was normalized with the housekeeping gene β-actin, and the relative expression levels were calculated using the 2 -ΔΔCt method.

Declarations

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Availability of data and materials

The whole genome sequences and transcriptome data of *G. citri-aurantii* are available at the National Center For Biotechnology Information (NCBI) under PRJNA578961. The genome sequences of *G. candidum* could be found on NCBI (PRJNB5752).

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

CAL and JZ planned and designed the research. JZ, YDZ, ZW, ZHT, FY and JXL analysed genomic and transcriptomics data. JZ wrote the manuscript. All authors read, revised and approved the manuscript.

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Not applicable

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Tables

Due to technical limitations, Table 1 is only available as a download in the supplemental files section.

Figures

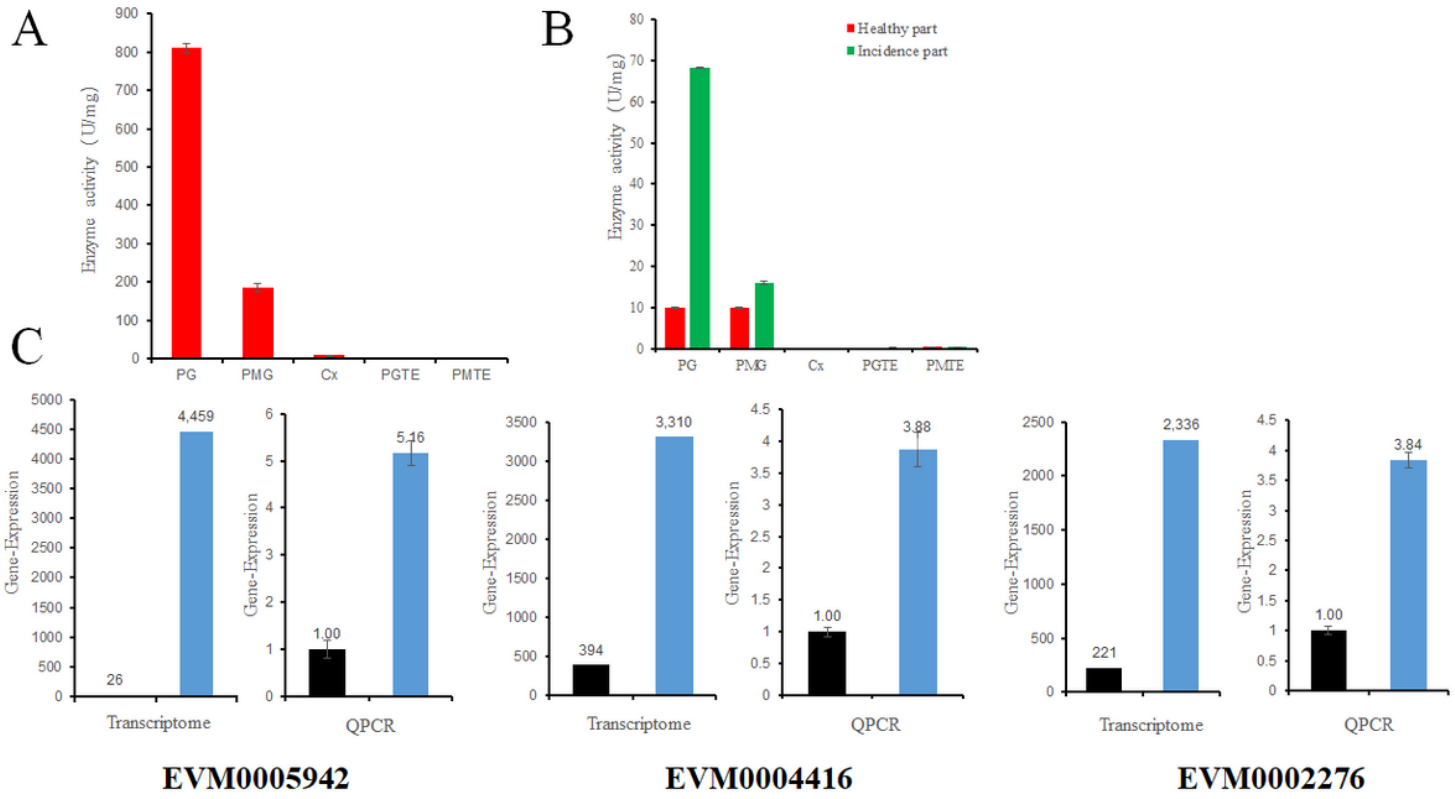

Figure 1

Determination of cell wall-degrading enzyme activity of G. citri-aurantii and expression analysis of three PG genes. A. Activity of cell wall degrading enzymes produced by G. citri-aurantii in vitro; B. Activity of cell wall degrading enzymes produced by incidence and healthy part; C. Expression analysis of 3 PG genes after infection with citrus. The mRNA abundance was normalized using the housekeeping gene actin, and the relative expression levels were calculated using the 2-ΔΔCt method. Three biological replicates were performed.

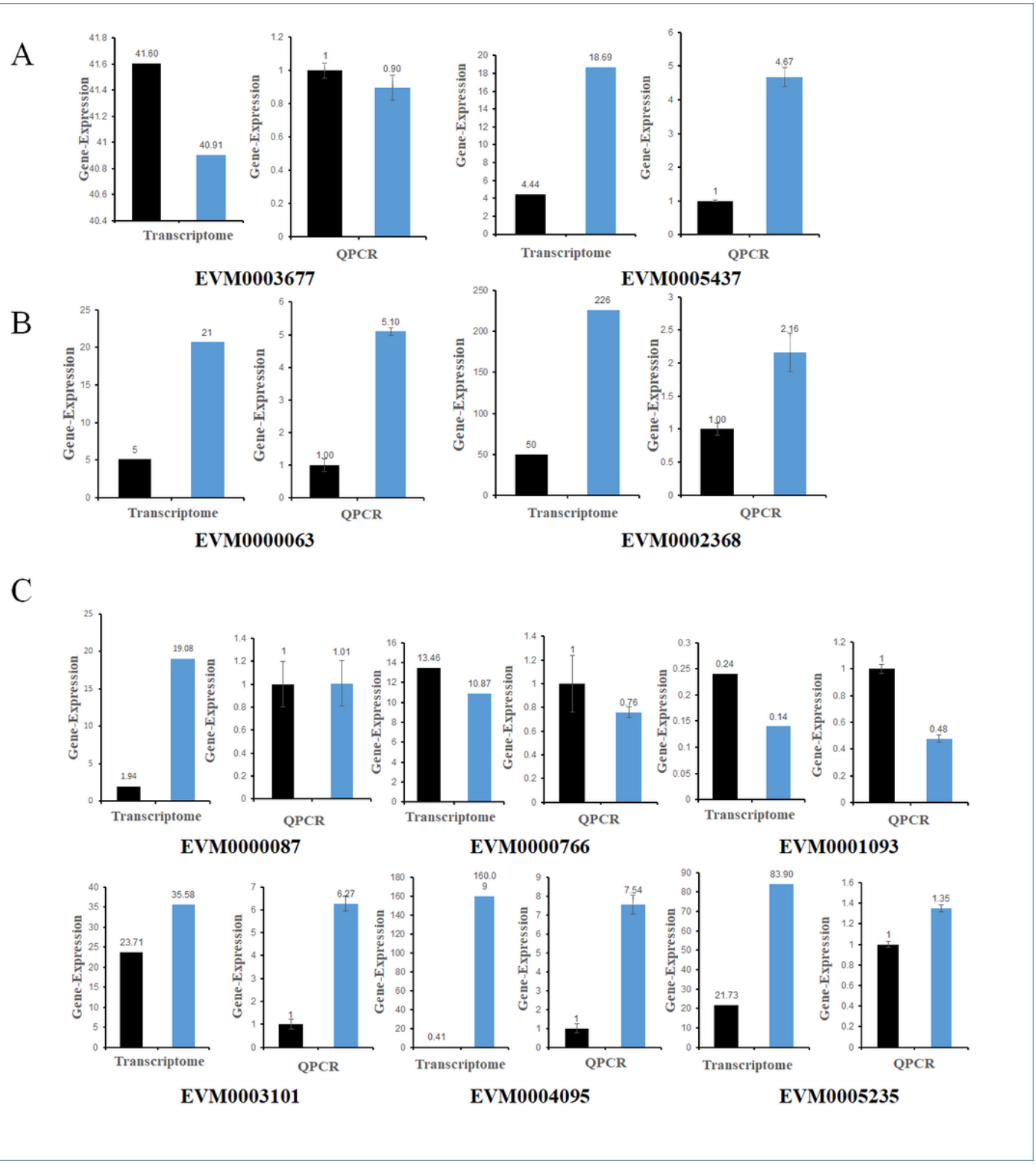

Figure 2

Expression analysis of chosen genes of the ABC family, MFS family and MATE family genes. A. Expression analysis of chosen transporter genes of ABC family genes in AY1-68 after albesilate treatment; B. Expression analysis of chosen transporters of MATE family genes in AY-1 and AY1-68; C. Expression analysis of chosen transporter genes of MFS family genes in AY1-68 after albesilate

treatment The mRNA abundance was normalized using the 18s housekeeping gene, and the relative expression levels were calculated using the 2-ΔΔCt method. Three biological replicates were performed.

Supplementary Files

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- Table1.png